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HONEYCOMBED STRUCTURE AND THE METHOD OF ITS MANUFACTURE, AS WELL AS A FILM AND CELL CULTURE SUBSTRATE THAT UTILIZES THIS STRUCTURE

[Hanikamu kozotai oyobi sono choseihoho, narabini sono kozotai wo mochiita firumu oyobi saibo baiyo kizai]

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Claims

1. A honeycomb structure obtained by casting a solution of a hydrophobic polymer in an organic solvent comprising 50-99% w/w of a biodegradable polymer and 50-1% w/w of an amphipathic polymer onto a plate in air at 50-95% relative humidity, and causing said organic solvent to gradually escape while simultaneously causing dropwise condensation on said cast liquid surface, and causing the fine water droplets formed by said dropwise condensation to be evaporated.

2. A film comprising the honeycomb structure according to Claim 1.

3. The honeycomb structure according to Claim 1 wherein the aforementioned biodegradable polymer is an aliphatic polyester.

4. A cell culture substrate comprising the honeycomb structure according to Claim 1.

5. The cell culture substrate according to Claim 4 wherein the diameter of the aforementioned honeycomb structure is 0.1-10 μm.

6. A method for preparing a honeycomb structure, wherein a hydrophobic organic solvent containing 50-99% biodegradable polymer and 1-50% amphipathic polymer is cast onto a substrate in air at 50-95% relative humidity, and said organic solvent is allowed to escape gradually while simultaneously dropwise condensation is caused upon said cast liquid surface.

Detailed Explanation of the invention

[0001]

Technical field of the invention

The present invention pertains to a foundation for cell culture that provides a better foundation for cell and tissue engineering, which are flourishing research subjects in the fields of medicine and agriculture in recent years. More specifically, the present invention offers a foot-hold for occasions

when cells are to form a 3-dimensional organization, and/or a substrate upon which cells may be cultured.

[0002]

Prior Art

It is known that, concerning the interaction between cells and substrates, cells are receptive to being influenced not only by the chemical characteristics of the substrate surface, but also to subtleties of its shape. From the point of view of tissue engineering and the aim to control cell functions, for example, it is important to finish both the chemical characteristics and the fine structure of the substrate surface that comes into contact with the cell. It is known that a great influence is imposed on such things as, for example, cell growth by the fine surface structure, the implementation of manufacturing fine contours on microspheres, the introduction of microgrooves onto the culture plate, size control of the cell contact surface by utilizing micropattern techniques utilized in the semiconductor industry, and other methods of finely finishing the surface.

[0003]

Concerning the establishment of a surface using these micropattern techniques, the present situation is one that embraces many problems, in that an extremely high-level technology is required, mass production is impossible, and costs are high, for example. A completely different surface patterning technology is also known in which a film is obtained having a honeycomb structure on a micron scale, by casting a dilute polymer solution that has a special structure under high humidity conditions. The present method is characterized by the fact that, in the manner of patterning, the economic characteristics are excellent.

[0004]

More specifically, an example is disclosed in Science, 1999, Volume 283, p. 373, wherein a polyphenylquinoline block polymer is used having a rod-coil diblock polymer comprising a hydrophilic block and a hydrophobic block, and another example in Nature, 1994, Volume 369, p. 387, wherein a diblock polymer is used comprising polyparaphenylene, which is a rigid block, and polystyrene. In this way, the prior art prepares a honeycomb structure by utilizing special polymers that have both a moiety with a strong self-aggregation capacity and a moiety that exhibits flexibility, dissolving these polymers in a hydrophobic organic solvent, and then casting this. On the other hand, the inventors have reported in Thin Solid Films, 1998, Volumes 327-329, p. 854, Supramolecular Science 1998, Volume 5, p. 331, and Molecular Crystals and Liquid Crystals 1998, Volume 322, p. 305, that an amphipathic polymer having both a hydrophilic acrylamide polymer as the main skeleton, a dodecyl group as a hydrophobic side chain, and either a lactose group or a carboxyl group as a hydrophilic side chain, or an ion complex of a quaternary long-chain alkyl ammonium salt and anionic polysaccharide such as dextran sulfate or heparin, gives a thin film having a honeycomb structure by a similar method.

[0005]

Nevertheless these polymers do not offer adequate functionality as cell culture substrates, because they have various defects such as poor self-assembly characteristics of the honeycomb structure obtained, and the collapse of honeycomb structures over time, for example.

[0006]

Problems to be solved by the invention

When cells are cultured for the purposes of cell or tissue engineering, a substrate that forms the foot-hold of the cell is required and, as mentioned above, it is known that, concerning the interaction between cells and substrates, cells are receptive to being influenced not only by the chemical characteristics of the optimal surface, but also to subtleties of its shape. When control of the cell functions is the objective, it is important to design both the chemical characteristics of the substrate surface that comes into contact with the cell and the fine structure of the cell. Porous films that have a honeycomb structure offer a cell contact surface with a honeycomb pattern, and it has been shown that the porous structure forms a nutrient supply route, and there is access for the cell support substrate.

[0007]

It is thought that 1 utilization method, if the cell is organized on a film base with a honeycomb structure, is in an artificial organ. In this case, due to the necessity of implanting the artificial organ into a body, it is desirable for the substrate to be absorbed into the living system over a long period of time. The materials until now that have given honeycomb structures have not been made from biodegradable materials such that a stable structure has been maintained for the period of time needed to culture the cells, and then degraded after this time. In other words, there is a requirement to use a biodegradable material suitable for deployment in medical applications such as artificial organs, that combines the honeycomb structure with cell engineering and cell culture technology.

[0008]

Means to solve the problem

As the result of conducting diligent research to address the issues and problems described above, the inventors discovered that economical manufacturing was possible by combining in suitable proportions a biodegradable polymer with an amphipathic polymer, giving a structurally stable honeycomb structure which is also autonomous. In other words, the invention was accomplished as follows.

[0009]

(1) A honeycomb structure obtained by casting a solution of a hydrophobic polymer in an organic solvent comprising 50-99% w/w of a biodegradable polymer and 50-1% w/w of an amphipathic polymer onto a plate in air at 50-95% relative humidity, and causing said organic solvent to gradually escape while simultaneously causing dropwise condensation on said cast liquid surface, and causing the fine water droplets formed by said dropwise condensation to be evaporated.

(2) A film comprising the honeycomb structure according to (1).

(3) The honeycomb structure according to (3) [sic] wherein the aforementioned biodegradable polymer is an aliphatic polyester.

(4) A cell culture substrate comprising the honeycomb structure according to (1).

(5) The cell culture substrate according to (4) wherein the diameter of the aforementioned honeycomb structure is 0.1-10 μm.

(6) A method for preparing a honeycomb structure, wherein a hydrophobic organic solvent containing 50-99% biodegradable polymer and 1-50% amphipathic polymer is cast onto a substrate in air at 50-95% relative humidity, and said organic solvent is allowed to escape gradually while simultaneously dropwise condensation is caused upon said cast liquid surface.

[0010]

Embodiment of the Invention

The following are preferred as the biodegradable polymer in the present invention, in terms of their solubility in an organic solvent: polyhydroxylactic acid, polycaprolactone, polyethylene adipate, polybutylene adipate, and other biodegradable polyesters; as well as polybutylene carbonate, polyethylene carbonate, and other aliphatic polycarbonates. Of these, polylactic acid and polycaprolactone are preferred in terms of ready availability and cost, for example.

[0011]

With regards to the amphipathic polymer used in the present invention, in terms of the necessity that it not be toxic and in consideration of the fact that it will be used in a cell culture substrate, it is preferable to use an amphipathic polymer having a combination of carboxyl or lactose groups as the hydrophilic side chains and a dodecyl group as the hydrophobic side chain, with the main skeleton being an acrylamide polymer, a polyethylene glycol/polypropylene glycol block copolymer, or an amphipathic polymer having as the hydrophilic group a water-soluble protein such as albumin, collagen, gelatin, or an ion complex with a long-chain alkyl ammonium salt and an anionic nucleic acid higher polymer such as RNA or DNA, dextran sulfate or heparin, for example.

[0012]

When manufacturing the invented honeycomb structure, it is necessary that the organic solvent to be used is not water soluble, because it is necessary to cause the formation of fine water droplets on the liquid polymer solution. The following may be mentioned as examples: halogen organic solvents such as

chloroform and methylene chloride; aromatic hydrocarbons such as benzene, toluene, and xylene; esters such as ethyl acetate and butyl acetate; non-water-soluble ketones such as methyl isobutyl ketone, and carbon disulfide, for example. It does not matter whether these organic solvents are used alone or as mixtures. The polymer concentration of both the biodegradable and the amphipathic polymers to be dissolved is from 0.01-10 wt%, preferably from 0.05-5 wt%. It is unfavorable for the polymer concentration to be less than 0.01 wt%, because the mechanical strength of the film obtained will be insufficient. If it exceeds 10 wt%, the polymer concentration is then too high, and an adequate honeycomb structure will not be obtained. The composition ratio of biodegradable and amphipathic polymers is to be from 99:1 through 50:50 (w/w). If the ratio of the amphipathic polymer is less than 1, it will be impossible to obtain a homogeneous honeycomb structure. It is undesirable for said ratio to exceed 50, because this is detrimental to the stability of the honeycomb structure obtained, and to mechanical stability in particular.

[0013]

In the present invention, in which said polymer solution in an organic solvent is cast onto a substrate to prepare a honeycomb structure, the substrate for this purpose may be glass, metal, a silicon wafer, and other inorganic materials; a polymer with excellent resistance to organic solvents such as polypropylene, polyethylene, or polyether ketone; or a liquid such as water, liquid paraffin, or liquid polyether, for example. Of these, the use of water as the substrate is highly suitable because in this case the self-assembly which is a characteristic of said honeycomb structure is used to good advantage, so said structure can be easily extracted independently from the substrate..

[0014]

The mechanism by which the honeycomb structure is formed in the present invention is thought to be as follows. When the hydrophobic organic solvent evaporates, the temperature of the cast film surface drops due to loss of the latent heat, the fine water droplets condense and adhere to the polymer solution surface. The hydrophilic moiety in the polymer solution acts such that the surface tension between the water and the hydrophobic organic solvent is reduced, therefore when the fine water droplets are about to condense and form into a clump, stabilization occurs. As the solvent continues to evaporate, hexagon-shaped liquid droplets become lined up in a densely packed formation and finally the water escapes, leaving the polymer in a shape that is arranged in a regular honeycomb pattern. Therefore it is desirable that the environment for manufacturing said film be in the range of 50-95% relative humidity. If the relative humidity is less than 50%, the dropwise condensation on the cast film will be insufficient, but more than 95% is undesirable because then it become difficult to control the environment. The sizes of the honeycomb structures made in this way are from 0.1-10 μm one by one (individually), and within this range of sizes they can be suitably used as cell culture substrates.

[0015]

The following statements explain the present invention in greater detail by reference to application examples, but the present invention is not limited to these in any way.

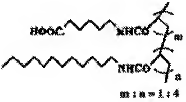
[0016]

<u>Application Examples 1-3</u>

A honeycomb structure was prepared by mixing a chloroform solution ((1.0 g/L) poly-L-lactic acid (molecular weight 85,000-160,000) and a benzene solution (1.0 g/L) Cap in structure 1 in the

proportions of 1:1, 4:1, and 8:1, casting this on a glass substrate, and allowing this to stand under conditions of room temperature and 80% humidity, thus allowing the solvent to escape gradually. Optical microphotographs of the structures obtained are shown in Figure 1. These films may be picked up with forceps, and the fact that they are self-supporting was demonstrated.

Structure 1



Structural formula of Cap

[0017]

<u>Application Example 4</u>

A collapsed film was prepared by placing Milli-Q water (40 mL) in a Petri dish (9.3 cm i.d.), mixing a chloroform solution of (1.0 g/L) poly-L-lactic acid (molecular weight 85,000-160,000) and a benzene solution of (1.0 g/L) Cap, which is an amphipathic polymer, in the proportion of 8:1 (wt%), and deploying 20 μL of this on the water surface. Subsequently liquid drops were formed by causing 10 μL of said polymer solution to drip downwards, and to this was applied blown air at 80% relative humidity at a rate of 90 mL/min, thus forming a honeycomb structure. Said structure could be scooped up in a frame (diameter = 5 mm), and it was confirmed to be self-supporting.

[0018]

Comparative Example 1

An attempt was made to prepare a honeycomb structure by an operation identical to that of Application Example 1, using only a chloroform solution of ((1.0 g/L) poly-L-lactic acid (molecular weight 85,000-160,000). The results are shown in Figure 2 [sic; There is no Figure 2. The official amendment does not mention anything about this.], but the morphology of the film obtained was not homogeneous.

[0019]

Comparative Example 2

An attempt was made to prepare a honeycomb structure by an operation identical to that of Application Example 1, using only a Cap solution. In this example, the film broke up during the evaporation of fine water droplets, and it did not have self-supporting properties.

[0020]

Test Example 1

The honeycomb film obtained in Application Example 1 was placed on a glass plate coated with poly-HEMA, and endothelial cells (ECs) derived from bovine aorta were cultured on this, using IMDM [Iscove's modified Dulbecco's medium] as the medium, in a CO_2 incubator (CO_2 concentration-5%, temperature = 37°C, relative humidity = 80%). As a comparative example, ECs were directly seeded onto glass plates coated with pHEMA, and this was cultured under identical conditions. With the former, the cells made good contact and deployed, so it was demonstrated that a film having a honeycomb

structure functions as a foot-hold for cells. However, the ECs made no contact whatsoever with the latter.

[0021]

Effect

As explained above, the invented method makes it possible to easily prepare a regularly arrayed honeycomb structure having a biodegradable polymer as the main ingredient, and also makes it possible to offer a cell culture substrate and a film using this.

Brief description of the figure

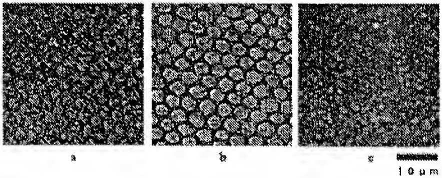


Figure 1: Optical microphotographs of the honeycomb structures with a = 1:1, b = 4:1, and c = 8:1 polylactic acid:Cap.